

ANGULAR LEAF-SPOT OF CUCUMBERS

By ERWIN F. SMITH, *Pathologist in Charge*, and MARY KATHERINE BRYAN, *Scientific Assistant, Laboratory of Plant Pathology, Bureau of Plant Industry*

INTRODUCTION

The angular leaf-spot of cucumbers (*Cucumis sativus*) has been known in the field for many years, but up to the present time no organism has been named as its cause, though it has been generally conceded to be of bacterial origin. The disease is characterized by the formation of numerous, often confluent, angular, dry, brown spots which by dropping out or tearing give the leaves a ragged appearance.

The literature on the subject, aside from mere notes on the occurrence of the disease scattered through pathological literature, consists of four papers by O. F. Burger, of Florida,¹ and a more recent Italian paper by Traverso.² Burger mentions the leaf-spot as preliminary to a more destructive fruit-rot, said to be due to the same organism. His description of the diseased leaves agrees with the appearance of leaves sent to the writers from Wisconsin, as well as with those obtained by them from other States, and with the leaf-spots which they obtained in Washington by pure-culture inoculations. A brief description of the causal organism is given in each of his papers, in one case with the group number according to the chart of the Society of American Bacteriologists. Burger's descriptions agree in the main except as to flagella and the diameter of his organism. In his earlier descriptions it is said to have polar flagella, but in the later ones it is reported to be peritrichiate. No name is given to the bacillus.

Traverso's paper is only a preliminary one, but it leaves no doubt as to the identity of the Italian and American disease. A motile, fluorescent, nonliquefying organism was isolated by him and inoculations were made with it, but no positive results were obtained (p. 459).

Who first reported this cucumber disease in the United States is uncertain; the senior writer has known it for 20 years, and several years ago (1904) plated out two yellow bacteria with which unsuccessful inoculations were made. Again, in 1907, at his suggestion, Mr. John R. Johnston, then of the Laboratory of Plant Pathology, made platings

¹ Burger, O. F. A new cucumber disease. *In Fla. Agr. Exp. Sta. Rpt.* [1911]/12, p. c-ci. 1913.

—— A bacterial rot of cucumbers. *In Phytopathology*, v. 3, no. 3, p. 169-170. 1913.

—— Bacterial rot of cucumbers. *In Fla. Agr. Exp. Sta. Rpt.* [1912]/13, p. xc-xciv, fig. 11-13. 1914.

—— Cucumber rot. *Fla. Agr. Exp. Sta. Bul.* 121, p. 97-109, fig. 37-42. 1914.

² Traverso, G. B. Sulla batteriosi del cetriolo in Italia. Nota preliminare. *Atti R. Accad. Lincei, Rend. Cl. Sci. Fis., Mat. e Nat.*, s. 5, v. 24, sem. 1, fasc. 5, p. 456-460. Apr. 5, 1915.

and isolated a yellow schizomycete with which unsuccessful inoculations were made on cucumbers in the Department greenhouses.

ISOLATION AND IDENTIFICATION OF ORGANISM

Specimens were sent to the Laboratory of Plant Pathology in August and September, 1914, from New York and Wisconsin. No complaint was made by the sender of any association with fruit-rot, either on his own initiative or when questioned.

The interior of the spots was found to be swarming with bacteria which on floating out on the slide showed active motility. Plates were poured from such spots and a white, motile, rod-shaped organism was isolated. Spray inoculations with subcultures from three colonies on these plates gave typical infections on young cucumber leaves, from which the organism was reisolated. Colonies (subcultures) from this reisolation were then used for spray inoculations, and again the typical disease was produced with great virulence.

In August, 1915, specimens were received from several localities in Wisconsin, Indiana, and New York and from Ontario, Canada. In each case the same organism was isolated in pure cultures and used to produce typical infections on cucumber leaves in the hothouse.

The organism causing the angular leaf-spot of cucumbers appears to be an undescribed form for which the specific name *lachrymans* is suggested on account of the tearlike drops of exudate from the spots in early stages of the disease. Its brief Latin diagnosis is as follows:

***Bacterium lachrymans*, sp. nov.**

Baculis cylindricis apicibus rotundatis, solitariis, saepe binis; baculis unis $0.8 \times 1-2\mu$; 1-5 flagellis polaribus mobilibus; aerobiis, asporis.

Habitat in foliis vivis Cucumeris sativi in maculis angularibus. Liquefacit gelatinam lente. Coloniae superficiales in agar-agar, rotundae, albae; coloniae juvenes habientes centra non-translucida, et margines translucidas cum lineis multis radiantibus. Lac sterile alkalinum et translucidum fit; casein non segregatur. Nitrum non redigitur; culturae in mediis cum saccharo sacchari et saccharo uvae acidae fiunt. Gas non facitur. Methodo Grami non coloratur.

The organism which the writers isolated from the Wisconsin cucumber leaves and have here designated "*Bacterium lachrymans*, n. sp." differed culturally in so many important respects from Burger's organism that all our cultural experiments were repeated. These repetitions, however, confirmed the differences, which are given in Table I.

While it is not doubted that Burger had this disease under observation, it is believed that the organism described by him is not its cause, but is rather the cause of a rapid soft-rot of the fruit. His organism, however, may be a wound parasite following injuries due to the organism here described.

TABLE I.—Differences between *Bacterium lachrymans* and Burger's cucumber organism

<i>Bacterium lachrymans.</i>	Burger's organism.
1. Polar flagellate.....	Peritrichiate flagellate.
2. Liquefies gelatin.....	Does not liquefy gelatin.
3. Clears milk without coagulation.....	Coagulates milk.
4. Strict aerobe (does not grow in closed end of fermentation tubes).	Facultative anaerobe (grows in closed end of fermentation tubes).
5. Forms acid from saccharose in fermentation tubes.	Does not form acid from saccharose in fermentation tubes.
6. Forms acid from dextrose in fermentation tubes.	Does not form acid from dextrose in fermentation tubes.
7. Not villous along line of stab in either agar or gelatin.	Villous along line of stab in both gelatin and agar.
8. Does not become yellow with age on sugar agars.	Becomes yellow with age on sugar agars.
9. Moderate indol formation.....	No indol formation.
10. Agar-plate surface colonies show many fine radiating lines.	Agar-plate colonies homogeneous in structure.
11. Does not cause soft-rot of cucumber fruits.	Causes a soft-rot of the fruit.
12. Surface colonies on agar plates are always round.	Agar colonies are round to ameboid.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

Mr. Frederick V. Rand, of this laboratory, by whom these specimens were collected, reported the disease in 1915, from the following localities:

MICHIGAN: Big Rapids, Muskegon, Grand Haven, Holland, Grand Rapids, and Hudsonville.

INDIANA: Plymouth, Monterey, Tyner, and Donaldson.

WISCONSIN: Racine, Portage, Ripon, Princeton, and Milwaukee.

NEW YORK: Constable, Malone, North Lawrence, and Long Island.

CANADA: Provinces of Ontario and Quebec.

In regard to the amount of injury caused by this disease, Mr. Rand says:

In most cases I found the angular leaf-spot causing a rather minor injury, but in an occasional field I found all the leaves back of the tips of the vines very badly shot-holed and presenting an exceedingly ragged appearance, such that serious injury to the crop must inevitably result. Last year this disease had done more damage than any other in the vicinity of Ripon, Wis.

This disease has also been reported recently from Maryland and several other Southern States.

Earlier the senior writer received specimens from Michigan, Wisconsin, Indiana, Connecticut, and the District of Columbia.

INOCULATION EXPERIMENTS

On October 26, 1914, young cucumber plants were sprayed in cages in the hothouse with water suspensions from young agar slants made from three colonies on the plates poured from diseased leaves. The plants were kept moist in the cages for 30 hours, then removed to the bench.

Five days later, water-soaked spots appeared on the leaves, and by November 3 there were typical browned spots on plants inoculated with each of the three colonies. These spots swarmed with bacteria. Poured plates on agar gave pure cultures of the same white organism. No further inoculations were made until April 30, 1915, when sprayings were again made in cages as before, using subcultures of colony No. 1, plated from a spot produced by the inoculations of October 26. The plants used in this case were of a common field variety and rather stunted but with sound leaves. Three days after the first spraying water-soaked spots appeared on the lower surface of the leaves, and by May 6 these had enlarged into the typical angular, dry, brown spots.

Another experiment on May 6, 1915, using perfectly healthy, free-growing Arlington white spine cucumber plants and subcultures from the same colony (No. 1) gave striking results. Several leaves showed tiny water-soaked areas on the second day, and all the leaves were typically and badly spotted by the sixth or seventh day. In this stage the spots were one-fourth to three-fourths of an inch in diameter, angular, following the larger veins, and water-soaked (translucent), not dry. In the early morning drops of moisture (exudate) swarming with bacteria were found hanging on the lower surface of such spots (Pl. XLV, fig. 1). Pure cultures of the causal organism were obtained by plating from one of these drops. On the following day, or even later on the same day, white films (bacterial crusts) replaced the drops (Pl. XLIII, fig. 1). The appearance of infected leaves at the end of 12 to 14 days, when the diseased areas have become dry and begin to drop out, is shown in Plate XLIII, figure 2.

As the young unsprayed leaves developed on these plants, they became naturally infected; and in three cases the stems and petioles of this young growth also became water-soaked, exuded drops of fluid (Pl. XLIV, X, X), and finally broke or bent over (Pl. XLV, fig. 2), ending the growth of the plant. The cracking open of stems in this stage of the disease is shown at X in Plate XLV, figure 2, and in detail in Plate XLV, figure 3.

On the green fruits up to the end of August, 1915, the writers were able, with one exception, to obtain within a week or 10 days (shipping time) only a local infection and a bacterial exudate such as that shown in Plate XLVI, figure 1—no general soft-rot. Even when the fruit (Pl. XLVI, fig. 1) was kept for another week at high temperatures (28° to 32° C.), it did not rot (Pl. XLVI, fig. 2). Altogether 15 such fruits were inoculated with virulent cultures, some on the vines and others in damp chambers.

Soft-rot occurred twice in young fruits (two-thirds grown) when placed in damp chambers after inoculation. In the first case (the exception referred to above), plates were poured from the soft interior of the one fruit thus affected. As only spreading fimbriate colonies were obtained, the soft-rot was attributed to an intruder, and no further studies were

made. Some months later (September, 1915) in a similar experiment two out of four inoculated fruits became soft-rotted. These fruits were from the market. All four showed the local gumming at the point of inoculation (needle pricks) after five days, while check pricks gave no gumming. Two days later two fruits began to soften, and the next day the whole interior was swarming with bacteria. Plates were poured from the interior of one of these fruits under sterile conditions, and again only spreading fimbriate colonies were obtained. Smears from these colonies stained by Van Ermengem's flagella stain gave rods with as many as 8 or 10 peritrichiate flagella. This organism grew well in the depths of agar stabs and curdled milk with reddening of litmus in milk. The other two inoculated fruits remained sound and after two weeks when cut open showed only a very local infection not extending much beyond the needle pricks in any direction.

Since the organism causing the leaf-spot is polar flagellate and aerobic, does not develop a fimbriate growth on agar, and does not curdle milk or redden litmus in milk, it is evident that this soft-rot was due to an intruder, which may have come from the surface of the fruits, since they were not sterilized, but only washed.

When these fruits became soft-rotted, the suspicion arose that possibly the softening and cracking of the stems and petioles (Pl. XLV, fig. 2) might also have been due to some unsuspected soft-rot organism. The inoculation experiments with *Bact. lachrymans* were therefore repeated on stems and petioles of free-growing cucumbers with the same result as before—i. e., softening and cracking of the younger stems and petioles. From one of these stems platings were made and *Bact. lachrymans* obtained in pure culture. At the same time several control inoculations were made on stems and petioles, using a subculture of the fimbriate, peritrichiate, soft-rot organism plated from one of the softened cucumbers above mentioned, but no rot occurred (four weeks). This organism, however, soft-rotted green cucumber fruits when inoculated by needle pricks.

Last of all, following the discovery of Traverso's paper, another set of inoculations was made on cucumber fruits. Six marketable green hothouse fruits were selected and inoculated with *Bact. lachrymans*. At the end of 10 days in culture dishes at temperatures varying from 24° to 30° C. all showed local gumming and infection about the needle wounds, but none of them developed any soft-rot (Pl. XLVI, fig. 3).

HISTOLOGY OF DISEASED LEAVES

Pieces of a leaf that showed spotting were fixed on the second day, embedded, sectioned, and stained. Stomatal infections were very numerous (Pl. XLVII, fig. 1). The bacteria gorged the opening of the stoma in some cases, as well as the cavity beneath it. Even at this early date the bacteria had spread in great numbers for some distance from the stoma, crowding apart or crushing the cells of the parenchyma and causing a slight swelling on the leaf (Pl. XLVII, fig. 2).

MORPHOLOGY AND PHYSIOLOGY OF BACTERIUM LACHRYMANS

MORPHOLOGICAL CHARACTERS

As it occurs in the plant and also on media the organism causing the disease is a short rod with rounded ends, single or in pairs (Pl. XLVIII, fig. 2 and 3), 0.8μ wide by 1 to 2μ long. On culture media it occurs singly or in pairs with a very decided constriction, and occasionally (in salted bouillons) in chains of as many as 12 or more individuals (Pl. XLVIII, fig. 1). No spores have been seen. Capsules are formed on agar (Pl. XLVIII, fig. 2), and in milk (Ribbert's stain). It is motile by means of 1 to 5 polar flagella (Pl. XLVIII, fig. 3). It is Gram-negative and is not acid-fast.

EFFECT OF DESICCATION

When drops from 24-hour peptone bouillon were placed on sterile covers in sterile Petri dishes and kept in the dark at room temperature, the organism was not killed by 21 days' drying, but it gave no growth when covers were dropped into suitable bouillon after 6 weeks' drying.

TEMPERATURE RELATIONS

The best growth was obtained at 25° to 27° C. There was no growth at 36° , though bouillon was weakly clouded at 35° C. Slow growth occurred at 1° in bouillon cultures (two weeks' time).

SENSITIVENESS TO SUNLIGHT

Agar plates, thin-sown, from an 8-day bouillon culture were exposed, bottom up on ice, to sunlight in June for 5, 10, and 15 minutes, one-half of each plate being protected from the light by several thicknesses of black paper. After five days' incubation numerous colonies appeared, and no difference was observed between the insolated and covered side on any of the six plates (but the colonies were not counted). Another test was made in September, 1915, with the following results:

The fluid used for inoculation consisted of one 3-mm. loop from a 24-hour bouillon culture into 10 c. c. of bouillon. Five plates were inoculated, each with one 2-mm. loop from this suspension. Five other plates were inoculated, each with one needle from this suspension. One plate from each lot was then half covered and exposed bottom up on ice for 5, 15, 30, 45, and 60 minutes, respectively. Result: All were killed by 45 and 60 minutes' exposure; three-fourths were killed by 30 minutes' exposure; one-third were killed by 15 minutes' exposure; and one-fourth were killed by 5 minutes' exposure.

When these results were obtained with the 24-hour bouillon, the experiment with the 8-day bouillon was repeated. Four agar plates were poured, one-half of each being exposed bottom up on ice, two for 15 minutes and two for 30 minutes, the sky being clear and the sun bright (October 12).

There was a marked reduction of colonies on the plates exposed for 15 minutes (estimated, 70 per cent), and almost complete absence of colonies on those exposed for 30 minutes (estimated, 95 per cent destroyed). The contradictory earlier result must therefore be attributed to a feebly actinic condition of the sky not visible to the naked eye.

SENSITIVENESS TO FREEZING

The organism is quite sensitive to freezing. A transfer was made to beef bouillon from a 5-day-old bouillon culture, shaken well and allowed to stand for five minutes. Plates were then poured with measured loops from this culture. The tube was then buried in salt and pounded ice, frozen solid and kept frozen for 15 minutes, after which it was thawed in cool water (five minutes required), shaken thoroughly, and used for a second set of plates, the loops being measured exactly as before. Two days after pouring the colonies were counted. There were one-ninth as many colonies after freezing as before freezing (Pl. XLVII, fig. 3). A longer incubation (five days) did not increase the number of colonies on the plates.

Thinking that five minutes might not have been long enough to obtain a uniform diffusion of the bacteria in the fluid, the experiment was repeated, allowing the tube to stand an hour with shaking before the plates were poured. The result was practically the same, nine-tenths of the bacteria being destroyed by the short freezing, the count being made on the fifth day.

CULTURAL CHARACTERS

AGAR-POURED PLATES.—On +15 peptone-beef agar at 23° C. surface colonies 2 days old are 1.5 to 2 mm. in diameter, round, smooth, shining, slightly convex, finely granular (under the compound microscope), with an opaque white center and a thin, transparent, entire margin. When 3 to 4 days old at 23° C. the largest measure 4 to 7 mm. in diameter and the white opaque center spreads in radiating lines into the thin margin (Pl. XLIX, fig. 1). At higher temperatures (27° to 30° C.) they reach this size in two to three days. Buried colonies are lenticular. Later (when 4 to 5 days old) the surface colonies lose their dense white center and dry down very thin and transparent and then show little or no trace of the radiating lines.

AGAR STABS.—Stabs in +15 peptone-beef agar when 2 days old at 23° C. show a raised, smooth, shining, white, transparent, surface growth 8 mm. in diameter. Growth is visible only along the upper one-third of the stab. This is granular, not villous.

Old cultures have a thin white growth completely covering the surface, and the agar is then frequently pale green, fluorescent.

AGAR SLANTS.—On slant agar, stroke cultures make a moderate, thin, white, transparent, smooth, shining growth, denser in the center. There is considerable white sediment in the V.

GELATIN PLATES.—Surface colonies on gelatin plates show a peculiar margin, best seen under low magnifications, with oblique light (Pl. XLIX, fig. 2). Liquefaction is slow (18° to 20° C.), and when the layer of gelatin is thin (10 c. c. to a plate) does not take place, as the medium soon becomes too dry for growth. On plates containing 20 c. c. of gelatin liquefaction began on the twelfth day and on the sixteenth day was complete, the colonies floating intact in the liquid gelatin.

GELATIN STABS.—At 15° to 18° C. in +10 peptone gelatin the surface growth after seven days is about 6 mm. in diameter, with a pit of liquefaction 2 mm. wide and 2 mm. deep. Stab growth is granular, not villous, fading out downward. As liquefaction progresses the upper part becomes stratiform, the lower part bluntly funnel-form (Pl. XLIX, fig. 3). Liquefaction progresses rather slowly but is complete within three to four weeks at the specified temperatures.

BEEF BOUILLON.—In +15 peptone-beef bouillon uniform clouding occurs within 24 hours. This clouding is weak to moderate, never strong. On the second day a membranous pellicle is formed, which fragments and falls readily on shaking. It is made up of a homogeneous mass of bacteria—i. e., free from pseudozoogloæ but containing a few short chains (10 or 12 individuals). Old cultures (4 to 6 weeks old) are often decidedly green fluorescent. The white precipitate breaks up readily on shaking and contains many small crystals.

POTATO CYLINDERS.—When inoculated from agar cultures growth on steamed potato cylinders in two days is moderate, spreading, creamy white, shining, and slimy. The part of the potato out of the water becomes slightly browned. Growth on potato soon ceases. After 10 days the color of the potato is completely changed, becoming a pale brownish hue, and the growth takes on a similar color (very pale brownish). Tested with alcohol iodine for starch, such cultures give a heavy dark-purple reaction, showing that there has been only a partial digestion of the starch (formation of amyloextrin). The cylinders are not softened.

MILK.—Inoculated milk clears slowly and without coagulation. Clearing begins within a week, and after two weeks tubes of it are translucent so that the outlines of a pencil back of the milk may be seen through it clearly. Cultures 1 month old are still clear but are then tawny olive,¹ with a darker rim where the milk has dried down.

LITMUS MILK.—Lavender-colored litmus milk begins to blue from the top downward on the second day and is completely blue by the third day, without a sign of coagulation or clearing. A decided creamy-white pellicle is formed.

After 10 days clearing begins and is complete in 20 days. Later the blue color bleaches out (reduction phenomena), beginning at the bottom, leaving the whole fluid a clear (translucent) brown. At no time is there any reddening of the litmus or any coagulation of the milk; nor are any crystals formed in it.

FERMENTATION TUBES.—The tests in fermentation tubes were made in water containing 2 per cent of Witte's peptone, to which was added 2 per cent of the carbon compound to be tested—namely, saccharose, dextrose, lactose, maltose, glycerin, and mannit. Clouding occurred in the open end of each on the second day, heaviest in the tubes containing saccharose and dextrose, but the closed end in every case remained clear, with a distinct line across the inner part of the U. When 5 days old they were tested with neutral litmus paper. Saccharose and dextrose gave a decidedly acid reaction, while all the others were neutral. When 20 days old the saccharose and dextrose were still acid and the others weakly alkaline. No gas was formed and no growth occurred in the closed end of any.

No gas was formed in fermentation tubes containing sterile milk; nor was there any separation of the curd. The milk in the open end cleared gradually, while that in the closed end remained unchanged. The litmus reaction was alkaline in the open end.

Nitrate bouillon in fermentation tubes gave a good clouding in the open end, none in the closed end, no gas, and no nitrate reduction. A decided alkaline reaction was obtained with neutral litmus paper.

TOLERATION OF SODIUM CHLORID.—Neutral peptone-beef bouillons containing 2, 5, 6, and 7 per cent of chemically pure sodium chlorid, respectively, were inoculated from young bouillon cultures. Growth was retarded by 2 per cent of sodium chlorid

¹Ridgway, Robert. *A nomenclature of colors* . . . 129 p., 17 pl. (partly col.). Boston, 1886.

and inhibited by all the other strengths. The experiment was repeated using 2, 3, and 4 per cent of sodium chlorid. Again, the 2 per cent retarded growth (clouding on the fourth day). Checks clouded after 24 hours. Growth appeared in the 3 per cent after 12 days, but there was no growth in the 4 per cent even at the end of four weeks. In both 2 per cent and 3 per cent the growth was scanty and flocculent, composed largely of chains (Pl. XLVIII, fig. 1), especially in the 3 per cent solution.

TOLERATION OF ACIDS.—Neutral bouillon containing 0.1, 0.2, and 0.3 per cent, respectively, of malic acid, tartaric acid, and citric acid was used. After three days the 0.1 per cent cultures of all three acids were well clouded; the 0.2 per cent malic and tartaric acids were all moderately clouded, while the 0.2 per cent citric acid showed no growth. None of the 0.3 per cent cultures were clouded. After three weeks the 0.2 per cent citric acid was well clouded, but in no case did the 0.3 per cent cultures show any growth. The cultures were watched for five weeks.

TOLERATION OF ALKALI.—The organism is quite sensitive to alkali. Peptonized beef bouillons titrating, according to Fuller's scale, +25, +20, +10, +5, 0, -5, -20, and -30, were inoculated from a 4-day bouillon culture, using a carefully measured 3-mm. loop for each tube. After 24 hours all showed growth except the -20 and -30. Heaviest growth occurred in the +25, weakest growth in the -5, which was flocculent instead of clouded. Five days later the same relative growth was evident throughout the series, but the -5 had become clouded and the -20 weakly flocculent. The -30 remained clear. After two weeks there was moderate growth in the -20, but none in the -30. The alkali used was sodium hydrate.

USCHINSKY'S SOLUTION.—In Uschinsky's solution growth is heavy, with a heavy membranous pellicle which falls readily as a whole. Greening of the media begins at the top on the second or third day and proceeds rapidly downward until the whole is a decided pale apple green. The medium does not become viscid.

FERMI'S SOLUTION.—At the end of 10 days a fine green fluorescence like that in Uschinsky's solution is visible. No fluorescence appeared in tubes of Cohn's solution inoculated on the same date for comparison.

COHN'S SOLUTION.—There is good clouding, heaviest near the top, but without a pellicle. Numerous floating crystals occur and the white precipitate is dotted with crystals. No greening occurs.

SUGAR AGARS.—No yellowing occurred on any of the sugar agars used. Cultures were made on beef-peptone agars containing, respectively, 2 per cent of saccharose, maltose, and dextrose, and in sugar agar without beef—i. e., containing only peptone and saccharose. The cultures were watched for eight weeks, during which time they remained white.

DOLT'S SYNTHETIC AGAR.¹—Growth is abundant, covering the surface on the third day with a thin pink layer. Reddening of the dark agar begins on the second or third day; and after 10 days the color is changed throughout, although the lower half has not lost completely its purplish hue.

BOUILLON OVER CHLOROFORM.—Growth is not retarded in unshaken tubes of peptone-beef bouillon to which 5 c. c. of chloroform have been added.

REDUCTION OF NITRATES.—Nitrates are not reduced. Five-day-old cultures in nitrate bouillon were tested by the addition to each of 1 c. c. of boiled starch water, 1 c. c. of potassium-iodid water, and 10 drops of sulphuric acid. There was no color reaction.

INDOL.—There is a weak indol production in 2 per cent peptone water and in peptonized Uschinsky's solution. Tests were made at the end of the fifth and tenth days by the addition of 1 c. c. of the standard sodium-nitrite solution and 10 drops of the sulphuric-acid water to each tube. No reaction appeared until the cultures were heated to 70° C., when a feeble but decided pink color appeared. The checks gave no pink reaction. A better reaction was obtained in peptone water containing 0.5 per cent of sodium chlorid (Dunham's solution)—about one-third that of *Bacillus coli*.

¹ Contains litmus, glycerin, milk sugar, and dibasic ammonium phosphate.

HYDROGEN SULPHID.—Strips of filter paper soaked in strong lead-acetate solution and dried were suspended over cultures in peptone-beef bouillon, milk, steamed potato, carrot, and turnip. No browning of the paper occurred within six weeks.

METHYLENE BLUE IN MILK.—Methylene blue is rapidly reduced. Cultures were made in milk containing 4 per cent of a 1 per cent solution of methylene blue. Bleaching begins on the second day and is complete or nearly so in six days, except for a pale-blue surface layer 2 to 4 mm. deep and a deep-blue rim and pellicle. This pellicle, when examined under the microscope, is seen to be composed of masses of bacteria that have taken up the stain. When shaken repeatedly, these bleached cultures regain their blue color.¹

BLOOD SERUM.—Stroke cultures on Loeffler's blood serum give a moderate, white, shining filiform growth 3 mm. wide. There is no liquefaction even after eight weeks and no color change in the substratum.

AEROBISM.—The organism appears to be strictly aerobic. It does not grow in the closed end of fermentation tubes with any carbon food tested. In agar stab cultures no growth occurs in the lower end of the stab. Cultures were also made by shaking an inoculated tube of melted agar, but no growth occurred more than 3 mm. below the surface. Stabs were made in agar, then 10 c. c. of melted agar poured on top. No growth occurred in the stab or at the junction point, but there was good growth on the exposed surface of the added agar.

LITMUS AGAR WITH SUGARS.—On litmus-lactose-agar stroke cultures there is moderate growth and no color change.

Stroke cultures on litmus-maltose agar give heavy growth, but do not alter the color.

On litmus-saccharose agar growth is heavy and the medium reddens, beginning at the thin upper end. The reddening begins on the second or third day and is complete on the fifteenth day.

Following the chart of the Society of American Bacteriologists, the group number is 211.23221*23.

EFFECT OF COPPER SULPHATE ON THE ORGANISM

Bouillon cultures 24 hours old were exposed to the action of chemically pure copper sulphate in the following manner. A dilution of copper sulphate (1 to 1,000) was made in a large Jena flask and allowed to stand overnight. After shaking thoroughly, further dilution was made again (in liter quantities) to 1 to 100,000 and 1 to 500,000. After these had been well shaken and had stood for an hour 10 c. c. of each were put into sterile test tubes and a loop of a well-clouded suspension from a 24-hour-old agar culture was added. Plates were poured after 5, 10, 20, and 30 minutes from each tube, using carefully measured loops. Checks were made by pouring plates with the same measured loops from a similar dilution in sterile water.

The plates were incubated at room temperature (27° to 30° C.). A colony count was made on the second day. Exposure to the 1 to 500,000 dilution gave no observed reduction of colonies, but the 1 to 100,000 destroyed nine-tenths of the organisms. The experiment was repeated with a strength of 1 to 50,000 of copper sulphate. All were killed at this exposure, while the check gave numerous colonies.

¹ The blue pigment is also absorbed by the bacteria from peptone water containing methylene blue.

*Nonchromogenic on most media, but green fluorescent in Uschinsky's solution, Fermi's solution, and old peptone-beef bouillon.

Some weeks later the experiment with copper sulphate was repeated. To liter quantities of distilled water in Jena flasks, chemically pure copper sulphate was added so as to obtain the following dilutions: 1 to 50,000; 1 to 100,000; and 1 to 500,000. Some hours after full solution, 10 c. c. of each dilution were pipetted into sterile test tubes and to each was added a 3-mm. loop from a heavily clouded water suspension made from a 24-hour agar slant culture. From each of these tubes three plates were then poured at the end of 5 minutes, and again three more at the end of 10 minutes. As a check, a 3-mm. loop of the cloudy bacterial suspension was added to 10 c. c. of distilled water and from this tube three plates were also poured. The agar for the first set of poured plates was seeded with a 3-mm. loop from the dilution tube, that for the second set with a 2-mm. loop, and that for the third set with a needle dipped one-half inch into the fluid. The results in colonies are given in Table II, the counts being made on the sixth day.

TABLE II.—Effect of copper sulphate on *Bacterium lachrymans*

Dilution used.	Checks.	Number of colonies of <i>Bacterium lachrymans</i> developing in—					
		1 to 50,000 copper sulphate.		1 to 100,000 copper sulphate.		1 to 500,000 copper sulphate.	
		5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.	5-minute exposure.	10-minute exposure.
Plate 1 (3-mm. loop)...	3, 844	78	45	118	55	3, 412	1, 756
Plate 2 (2-mm. loop)...	2, 296	27	16	29	44	2, 400	916
Plate 3 (needle).....	22	0	0	0	0	12	5

SUMMARY

(1) The angular leaf-spot of cucumbers is a widespread disease occurring in many of the Eastern and Middle Western States.

(2) It is characterized by angular brown spots which tear or drop out when dry, giving to the leaves a ragged appearance. In the early stages a bacterial exudate collects in drops on the lower surface during the night and dries whitish.

(3) Young stems and petioles may become soft-rotted or cracked open.

(4) A virulent outbreak often materially reduces the crop by destroying the needed active leaf surface.

(5) The spot is caused by *Bacterium lachrymans*, n. sp., which enters through stomata, no wounds being necessary. This organism is quite different from the one described by Burger¹ in his papers on cucumber rot. No direct connection has been found between the leaf-spot and the soft-rots of the fruit.

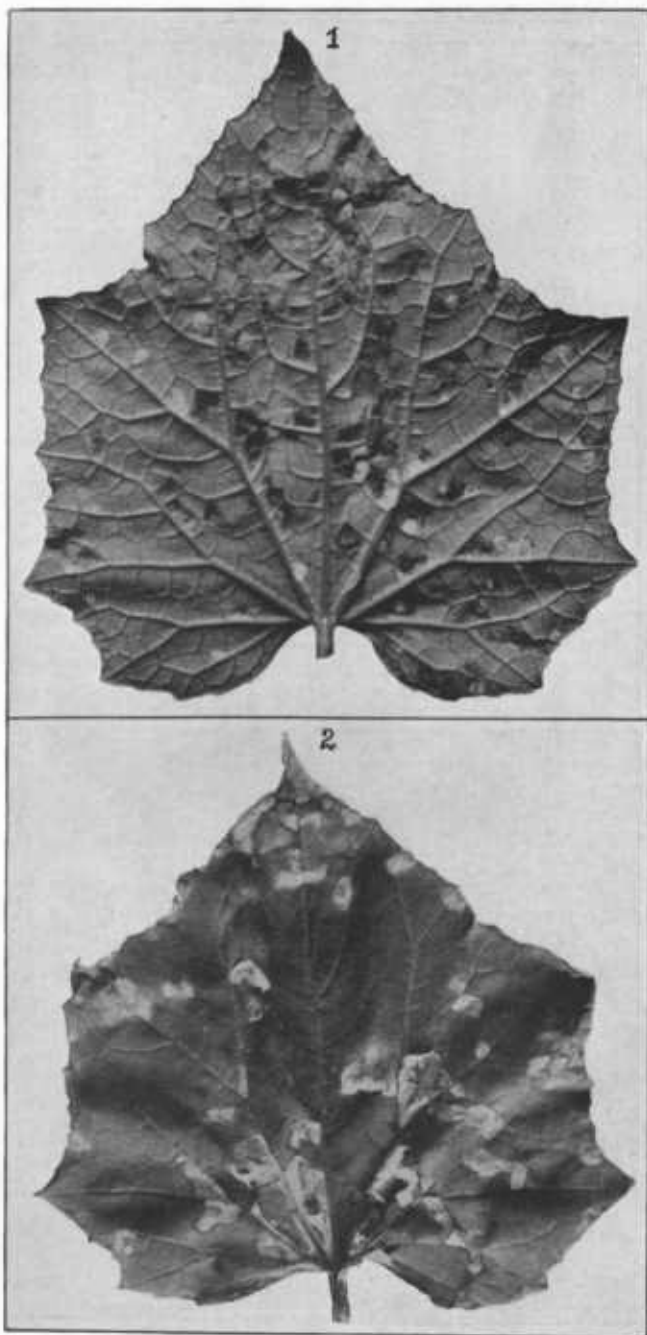
(6) Considering the results obtained in the laboratory with copper sulphate, it would seem that Bordeaux mixture properly applied is the remedy for this disease. Thorough field tests with it should at least be undertaken where the disease is troublesome.

¹ Burger, O. F. Op. cit.

PLATE XLIII

Fig. 1.—Cucumber leaf eight days after inoculation with *Bacterium lachrymans*. The bacterial exudate has now dried down into white crusts.

Fig. 2.—Cucumber leaf 12 days after spraying with *Bact. lachrymans*. Diseased tissue shriveled and spots falling out.



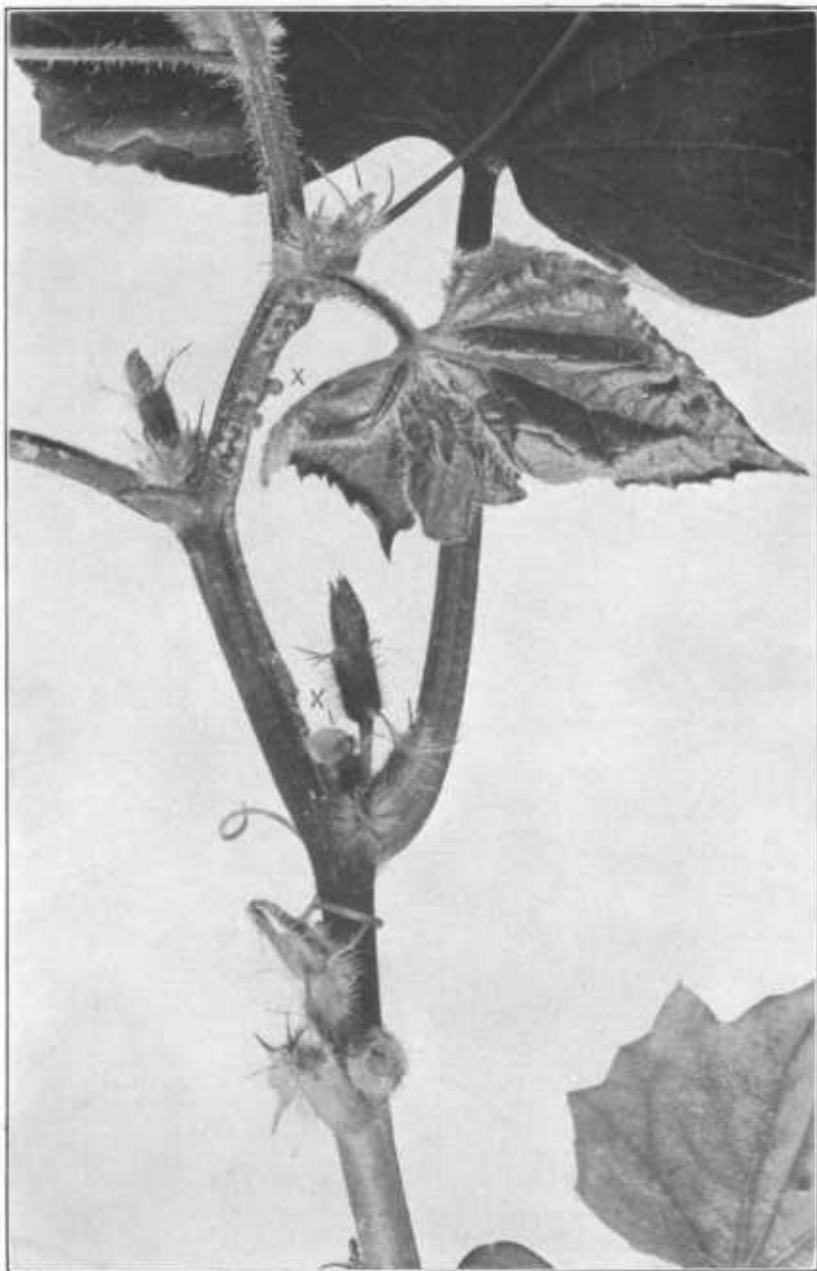


PLATE XLIV

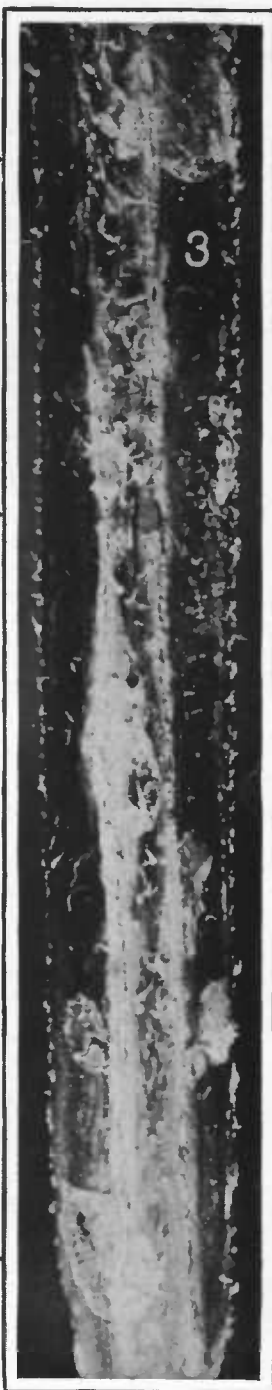
Cucumber stem diseased by *Bacterium lachrymans*. The white bacterial exudate may be seen at X, X. Photographed 14 days after spraying.

PLATE XLV

Fig. 1.—Fragment of a cucumber leaf showing angular leaf-spots due to pure-culture inoculation with *Bacterium lachrymans*. Time, six days. The glistening tearlike exudate can be seen in a number of places. $\times 2$.

Fig. 2.—Cucumber plant 18 days after spraying with *Bact. lachrymans*. Upper part of stem softened and shriveled. Lower part as at X with canker-like cracks which show bacterial exudate.

Fig. 3.—Stem at X in figure 2 enlarged to show bacterial lesions.



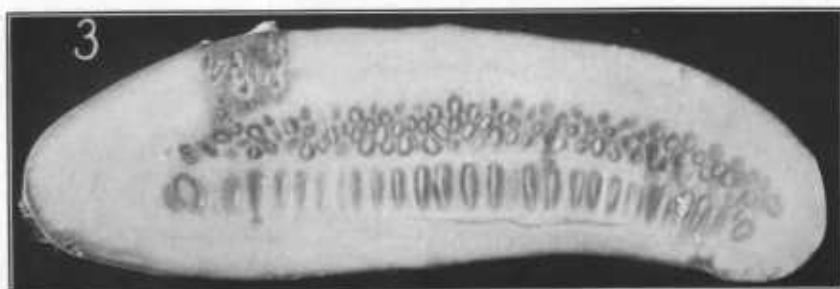
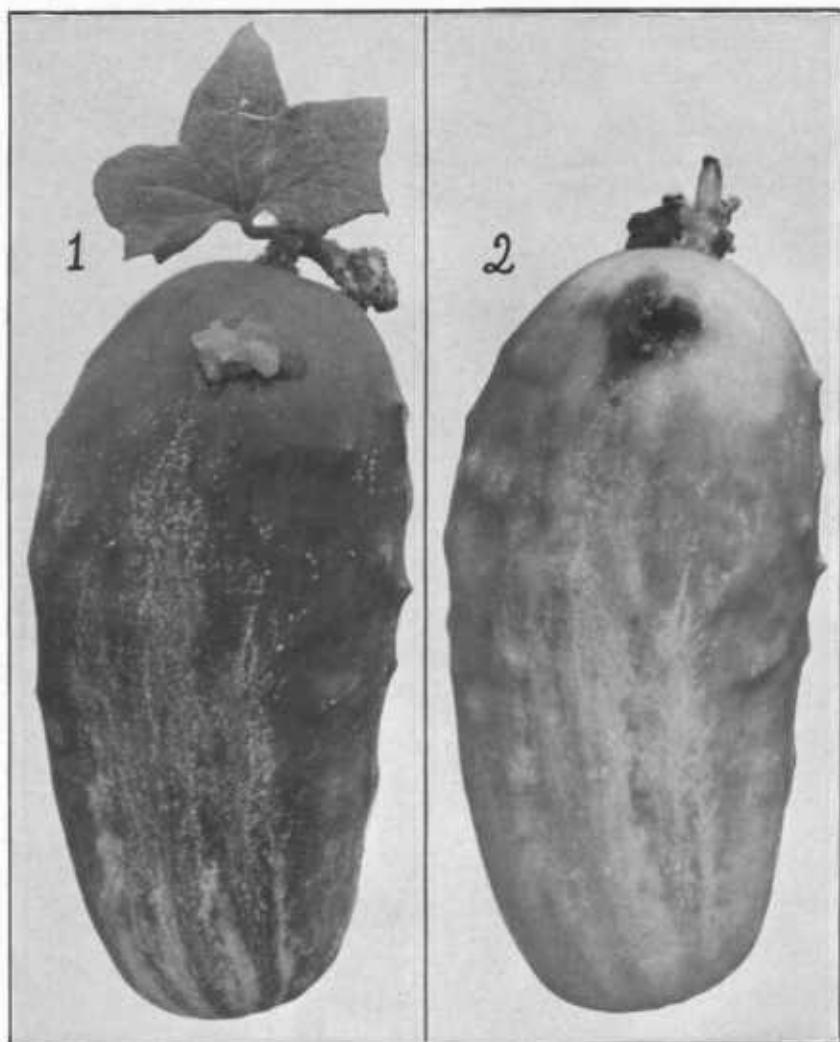


PLATE XLVI

Fig. 1.—Green cucumber fruit photographed six days after inoculation with *Bacterium lachrymans*. There is an exudate at the point inoculated (upper part of fruit), while the remainder of the fruit is sound.

Fig. 2.—Same fruit as shown in figure 1, but at the end of 12 days. The fruit, which was slowly ripening, was still sound both externally and within, except at the point inoculated.

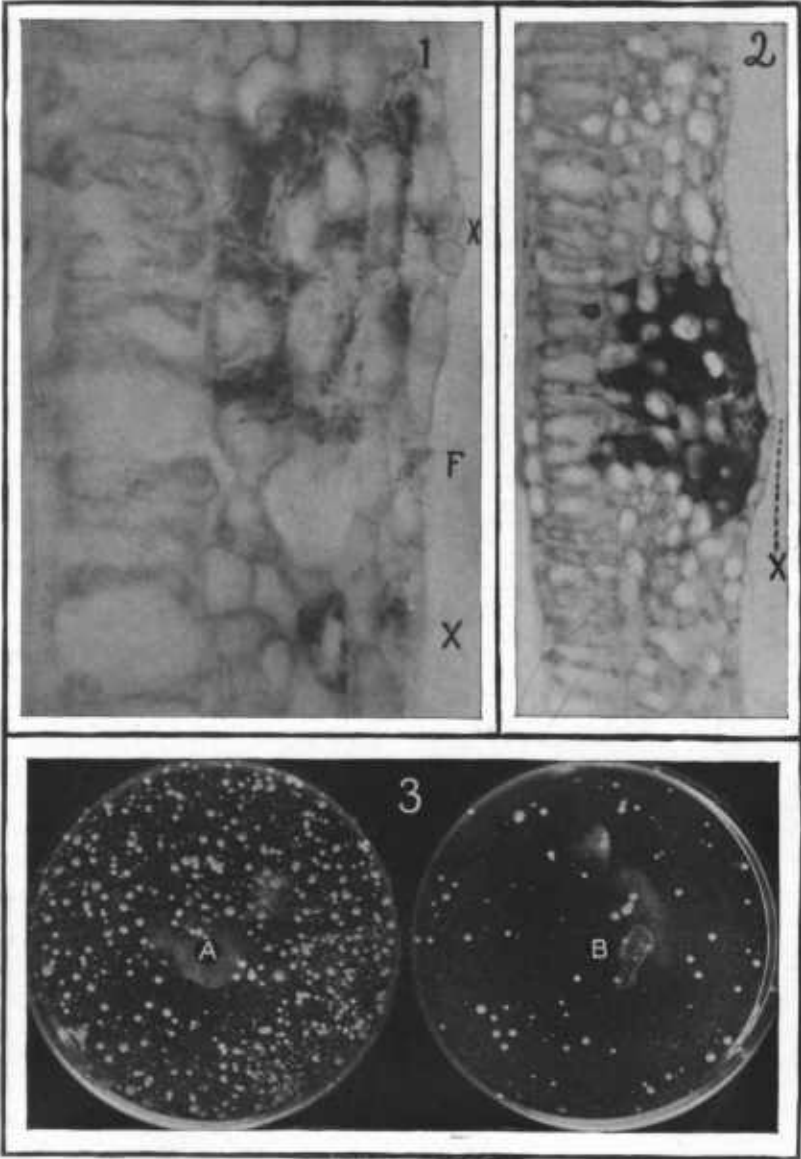
Fig. 3.—Section of green cucumber fruit 10 days after inoculation with *Bact. lachrymans* (6 days at 24° and 4 days at 30° C.). Not from the same series as figures 1 and 2. Tissue decayed only in the vicinity of the needle wounds.

PLATE XLVII

Fig. 1.—Cross section of a cucumber leaf, showing two stomatal infections (X, X). At *F* there is a third stoma whose chamber is free from bacteria. Stained with carbol fuchsin. $\times 1,000$, nearly.

Fig. 2.—Cross section of cucumber leaf showing a dense bacterial infection due to *Bacterium lachrymans*. Stoma at X. Moderate magnification. Carbol-fuchsin stain. Tissues pushed out.

Fig. 3.—*A*, Agar-poured plate from bouillon dilution of *Bact. lachrymans*; *B*, agar-poured plate made from same quantity of same bouillon as *A*, but after freezing 15 minutes.



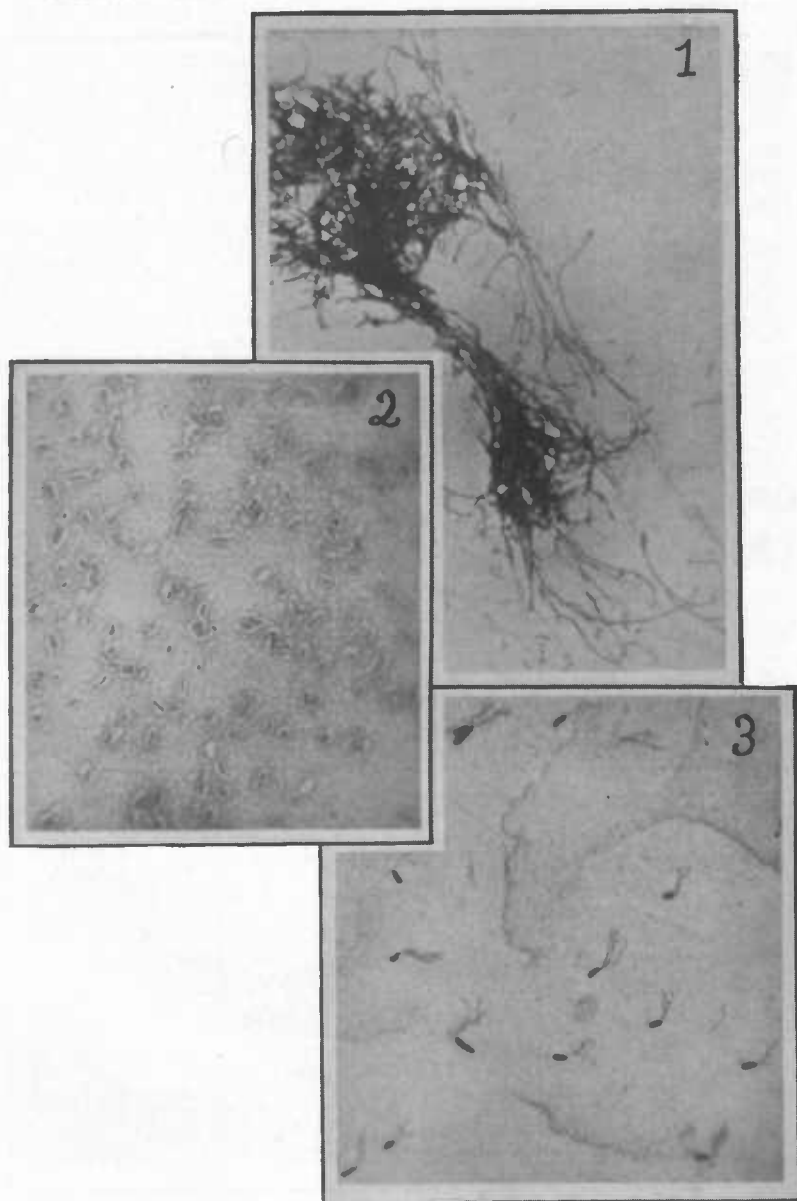


PLATE XLVIII

Fig. 1.—Chains of *Bacterium lachrymans* from 14-day-old culture in salted bouillon. Stained with carbol fuchsin. $\times 1,000$.

Fig. 2.—Capsules of *Bact. lachrymans* from young agar culture. Ribbert's capsule stain. $\times 1,000$.

Fig. 3.—Flagella of *Bact. lachrymans* from 24-hour-old agar slant. Stained by Van Ermengem's silver-nitrate method. $\times 1,000$.

PLATE XLIX

Fig. 1.—Young surface colonies of *Bacterium lachrymans* on agar poured plate, showing opaque center and lines radiating into the thinner margin. $\times 14$.

Fig. 2.—Surface colonies of *Bact. lachrymans* on gelatin poured plate. Photographed to show characteristic margin. $\times 14$.

Fig. 3.—Gelatin stab culture of *Bact. lachrymans*, kept at 20° C. and photographed at the end of 12 days. Liquefaction confined to the top, but a discrete growth along the line of the stab nearly to the bottom of the tube.

